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THE EFFECT ON MICE OF MINUTE DOSES OF B. ANTHRACIS.*

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ONE occasionally meets with the statement that minute accidental inoculations with various microorganisms in amounts too small to cause a noticeable reaction may immunize or, possibly, hypersensitize to further inoculations. The object of the experiments given below has been to investigate this matter as regards mice and anthrax, making use of doses ranging from one spore or one bacillus upward; and, further, to obtain some data as regards infection, especially regarding the minimum lethal dose of this organism for mice under different conditions. Mice and anthrax were chosen because of the extreme susceptibility of the animal and the possibility of manipulating the microorganism in a virulent condition both in the vegetative and the spore form. This combination of extreme susceptibility and extreme virulence gives, perhaps, the most severe test in an investigation on immunity. Further, as regards infection, anthrax is an organism to which man and the lower animals are many times exposed in small subcutaneous doses, the majority of which, doubtless, never lead to infection; and it might be of interest to ascertain the effect, if any, of such doses on a susceptible species.

The same race of anthrax was used throughout the whole series and various dosage employed. The same dose was sometimes repeated over a considerable interval, and sometimes gradually increased or decreased in amount. For the most part virulent material taken directly from an animal was employed, though some series were carried out with spores or vegetative material grown in culture. It may be stated at the outset that results as regards the attainment of any degree of immunity or hypersensitiveness were negative.

The size of the dose was estimated under the microscope. Spores were counted, and each filament of the vegetative form measured with the eyepiece micrometer, except in relatively few cases, desig-

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nated below, where larger doses were estimated less closely. In the isolation and inoculation of material a special technic involving the use of capillary pipettes was employed. This technic has been described in previous papers,[1] and only the special part of it developed in connection with this paper, the inoculation technic, need be described here.

Droplets containing single elements, or small groups of elements, were first isolated. Then with a fresh pipette containing a quantity of sterile physiological salt solution, the required dose was taken up. Before removing the pipette from the apparatus its tip was brought into contact with a large hanging drop of sterile salt solution suspended under the cover, and enough liquid admitted to wash the bacteria some distance back from the tip of the pipette. This is done to prevent the loss of the dose by the breaking off of the more delicate part of the tip in inoculating. A small portion of this tip may be purposely broken off before inoculating, since both the penetration of the skin and the discharge of the liquid are thus made easier. When the tip is inserted into the subcutaneous tissues of the animal, the contents are forced in by blowing into the rubber tube attached to the pipette. The liquid already in the pipette before taking up the dosage material serves to wash the bacteria out of the pipette. A new pipette is made for each inoculation.

For making inoculation pipettes a tough glass and tubing of somewhat thicker wall than is necessary for ordinary pipettes are used, and the portion just above the capillary tip is made somewhat thicker. The pipette is held in the right hand between the first finger and the thumb, and the second finger is extended to the bend in the pipette in order to press the tip through the skin. The mice were held in a short cylindrical holder, the tail brought through a small opening in a cloth placed over the open end of the holder, and, together with the edges of the cloth, held firmly to the side of the cylinder by means of a strong rubber band. The mouse could then be held in any desired position, the curve of the tail near the body making a convenient holding place for the left hand, leaving the right free for manipulating the pipette. Inoculations were made under low magnification furnished by large hand-lens supported on a jointed stand.

It is, of course, vital to the success of this technic that the bacteria pass into the animal with the inoculating fluid and none remain behind in the pipette. As a control a series of experiments was carried out in which the procedure was the same as in the mouse inoculations, except that the pipettes were discharged into suitable culture medium instead of into the animal. In one series 15 anthrax threads from cultures, five being short filaments containing one to three spores, were isolated and each thread drawn into a separate pipette and discharged into a separate test tube containing broth. All grew except three containing two, three, and two spores respectively. In another series, carried out by Dr. Hecker of this laboratory, 20 threads actively growing were manipulated in this manner and the emptied pipettes refilled with broth and incubated. The broth tubes or hanging drops receiving the discharge all showed growth while all the pipettes except two remained sterile. In a third series 15 single yeast cells, or attached groups of two to five, were treated in the same way as the anthrax filaments. Yeast cells were chosen because almost invariably viable when taken from actively growing cultures. Of these 15 all grew in the test tubes. So out of 50 experiments 47 exhibited growth in the test cultures. All experiments with actively growing threads or yeasts were positive, so it is not improbable that the failure of the three spore experiments was due to the lack of viability of the spores.

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[1] *Jour. Infect. Dis.*, 1908, 5, p. 380; *Kansas University Sci. Bull.*, 1907, 4, p. 3.

Further evidence of the reliability of the method is shown in portions of Table 1 where the material inoculated was especially virulent or the animals especially susceptible. In group 125, for instance, where all animals except one were inoculated with a single thread, four out of five were infected; and in the third inoculation of group 90 where larger doses were given, four out of four succumbed. The possibility of all or part of the inoculation material remaining behind cannot in every case be excluded, but the error due to this cause must be very small.

The chief sources of error in these experiments are, first, those inseparable from any animal experiments of this sort, the impossibility of exactly measuring the virulence of the inoculation material or of the varying resistance of the animals. These errors were eliminated to some extent by using a large number of animals. Over 160 mice were employed, not counting 23 which were inoculated with large initial doses for comparison or for furnishing inoculation material; and about 575 inoculations were made in the whole series. In the larger proportion of the inoculations material was taken directly from the blood or organs of an infected animal, in a majority of the cases after two or more passages through a series of animals. Material was taken from the animal and inoculated as soon as possible after death, and was kept cold until used, usually in a refrigerator. In some series material was inoculated within one or two hours after the death of the animal. In the culture and spore experiments nearly uniform conditions of growth were observed. Animals were kept, so far as possible, under conditions favorable to health.

A special source of error as regards the inoculation material is incident to the use of very minute doses. Here the chances of variation are greater than when larger doses are employed and there is the further danger of selecting dead or degenerate organisms for inoculation. In order to minimize this error selection was made of plump, healthy-looking bacteria, the organisms were not allowed to remain long isolated in droplets before inoculation, and droplets were protected from drying and strong light. Generally only a small number of animals were inoculated, rarely more than five, before fresh droplets were made, usually taken from the cold emulsion of organs or blood in salt solution. Inoculations were made as rapidly as possible. In some instances where small doses were given, seven or eight inoculations were made in an hour.

A test of the viability of the material inoculated was furnished by leaving in the droplets the bacilli remaining unused after a series of inoculations. The subsequent growth of a large percentage of these bacilli on the cover glass showed that they were viable. It is possible also that bacilli taken directly from an organ and inoculated into a mouse would be more likely to grow than when left in the culture medium used, a mixture of salt solution and mouse serum.

The possibility of inoculating aggressin, or some material capable of promoting infection, was kept in view, and in some earlier series the bacilli were washed by centrifuging before inoculation. But the dilution in salt solution, and the very small amount of this dilution inoculated reduce to a minimum the possibility of variation in results from this source. In the series where washed bacilli were used, the course of events was much the same as in cases where inoculated material was simply diluted with salt solution. It was demonstrated that a bacillus 6 μ long taken from the blood and washed in salt solution is capable of fatal infection.

All mice were inoculated at the same or approximately the same point—at one side of the root of the tail—and the dose was injected into the subcutaneous tissue, often somewhat beneath the skin itself. Since the point of the pipette could not always be brought into exactly the same place, some variation from this source as regards infection cannot be excluded. The lesion made by the very fine point of the pipette is so small as to be scarcely visible under the lens.

In Table 1 are given the results of experiments made with virulent vegetative material, taken, with a few exceptions, directly from the cadavers of infected animals. The exceptions are chiefly those in which the first one or two doses were made with some other material, either spores or culture material in the vegetative form. A few are included in which the material for one or two doses came from an infected guinea-pig or from infected insects. All these exceptions are indicated in the table.

Mice are ranged in groups separated in the table by horizontal lines. In each group are arranged those animals which received the same, or approximately the same, treatment during at least a part of the period of inoculations. This is especially true of the groups below 132. In these groups inoculations carried out on the same dates and with material from the same cadaver are for the most part found in one vertical column. The members of the first and second groups have less in common and are arranged together for convenience. In practically every case a given inoculation material was used only on one day.

For convenience in reference a group will be referred to by its first number: thus group 2 includes numbers 2, 3, and 4.

The unit in dosage is one linear micromillimeter. The number of threads inoculated, indicated by the Roman characters, is followed in the table by a number giving the sum of the lengths of these rods or threads as measured by the eyepiece micrometer. The intervals between doses are somewhat irregular in the earlier inoculations owing to interruptions in the work, but in the later ones they were for the most part between 10 and 20 days. A sufficient time was allowed for possible hypersensitiveness to develop, and to make the interval far exceed the usual interval intervening between the last inoculation and death from anthrax. In a number of the later inoculations the interval was shortened to seven to eleven days.

In a number of cases inoculated animals died from causes other than anthrax, though for the most part animals remained in good health over long periods of confinement in individual cages. The cages had for a base a glass jar partly filled with sawdust and cotton and, extending some six inches above this, an extension made of wire screen; so that the animals had roomy, well-ventilated quarters. It was observed that some animals did not do well immediately on removal to separate cages, especially in colder weather. To obviate a possible lowering of resistance from this cause animals in groups 125, 131, 139, 150, 171, and 181, aggregating nearly one-half of the total number in the table, were isolated some weeks before the first inoculation. The experiments extended over nearly one year, and no particular seasonal variation was observed.

It will be observed that the dose is made to increase slowly in groups 2, 83, 90, and some members of 13. It is made to increase rapidly in groups 171 and 181. It is made to remain nearly constant and relatively large in the large part of groups 19, 54, 71, 78, and 95. In some members of these groups an initial increase is followed by a slight decrease or the dose remains nearly constant. It is made to remain nearly constant and relatively small in groups 113, 125, 131, 139, 150, and 161. It decreases constantly in Nos. 65 and 95. In several groups the dose was made to increase rapidly at the end, and in groups 61, 64, 71, 78, 95, 181, and in Nos. 57, 58, and 59 a large initial dose of mouse or cockroach material was given. In group 181 an initial dose of mixt fresh and culture material was inoculated.

In none of these combinations do we find evidence of the establishment of any degree of immunity. Some mice resisted much longer than others and a few of these resistant ones were still surviving at the close of the experiments; but the resistance of these animals can well be explained by their greater natural immunity or by the size or condition of the animal without assuming the acquirement of artificial immunity. It will be observed that group 150, which consisted principally of large male mice, showed better resistance to the initial doses than the smaller animals in group 131, though the latter received smaller doses. Members of both lots had been isolated in individual cages for some weeks previous to the first inoculation. Also the mice of the more resistant groups 171 and 181 were older and in better condition than the mice of the same lot included in groups 113, 125, and 131. Further, it will be observed that the more resistant mice more often showed a decided

TABLE 1.

MICE INOCULATED WITH MATERIAL DIRECTLY FROM INFECTED ANIMALS.

Under species, *w*=white, *g*=gray. In weight column first number or designation=weight at beginning of a series; second=weight at autopsy or at end of series. If two numbers in the first column, lower=weight at some time during series. Roman numerals=number of filaments, Arabic=total number of micromillimeters in dose. Thus II=27 means two filaments aggregating 27 μ. In second line of dose description letters b, s, l, k, and st indicate blood, spleen, liver, kidney, and subcutaneous fluid respectively. If culture, 1 br, 1 ag, 2 ag, etc., indicate first broth, first agar, second agar, etc., cultures respectively. sp=spore. Numbers indicate number of mouse from which inoculating material was taken. Thus b 34=blood of No. 34, etc. ckr=cockroach, gp=guinea-pig. Following last dose +A=died of Anthrax, + ?=died cause unknown. Numbers in parentheses=number of mouse passages preceding material inoculated at fatal dose.

No.	Species	Weight in Grams	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days
2	w	med.	I=6 b 1	2	+A(1)									
3	w	med.–29.0	I=9 b 1	6	I=21 b 10	10	I=40 b 15	39	V=46 b 53	19	IV=50 b 16	15	V=51 b 82	9
3	w	(*Continued*)	+A(1)											
4	w	med.–20.0	I=30 b 1	7	I=18 b 9	9	I=40 b 15	39	VIII=52 b 53	16	III=65 b 63	18	III=102 b 82	3
4	w	(*Continued*)	+A(1)											
13	w	med.	I=9 b 10	10	II=27 b 15	4	+A(4)							
14	w	med.–22.0	I=7 b 10	10	II=27 b 15	39	VI=31 b 53	16	II=48 b 63	18	VIII=54 b 82	4	+A(1)	
16	w		I=6 b 9	48	II=13 b 53	16	II=42 b 63	2	+A(1)					
17	w	med.	I=21+1 sp 1 br 9	14	III=84+2 sp 1 br 13	42	10 sp b 14	2	+ ?					
18	w	med.	I=36+1 sp 1 br 9	14	IV=84+2 sp 1 br 13	33	VII=84 b 53	3	+A(3)					
34	g	small	I=12 b 15	3	+A(4)									
37	g	med.	II=57 b 15	42	I=21 b 18	16	II=27 b 16	15	+					
40	g	med.–15.5	II=57 b 34	36	III=57 b 53	19	VI=57 b 16	15	VI=73 b 82	3	+A(1)			

19	w	19.0			I=12 b 7	47	I=15 b 53	35	II=25 b 78	17	V=26 b 98	23	III=36 b 105	2
19	w	(*Continued*)	+A(1)											
27	w	med.–22.0			I=12 b gp	43	III=15 b 53	34	III=36 b 82	18	III=35 b 98	23	IV=48 b 105	4
27	w	(*Continued*)	+A(1)											
41	w	med.–24.7	Large am't degen. rods b 13	37	I=27 b 18	16	IV=35 b 16	15	VI=43 b 82	18	V=30 b 98	23	III=45 b 105	14
41		(*Continued*)	VI=45 b 110	28	V=40 b 24	14	V=40 b 125	18	III=41 b 26	16	III=43 s 156	24	IV=49 s 28	5
41	w	(*Continued*)	+A(1)											
45	w	29.0	III=93+3 sp 1 br 13	36	I=18 b 18	16	II=30 b 16	15	IV=43 b 82	18	VI=44 b 98	23	IV=57 b 105	14
45	w	(*Continued*)	X=63 b 110	4	+A(3)									
50	w	20.0			75–100 sp dried 10	33	V=51 b 18	31	IV=69 b 82	18	X=63 b 98	23	VI=75 b 105	5
50	w	(*Continued*)	+A(1)											
54	g	med.–15.9	I=9 b 11	74	VIII=47 b 27	14	VI=43 b 92	5	+A(4)					
55	g	med.–18.3	I=18 b 11	30	III=25 b 78	19	II=26 b 99	21	II=30 b 105	14	VI=42 b 110	28	VI=38 b 24	14
55	g	(*Continued*)	III=43 b 125	5	+A(5)									
56	g	med.–17	I=9 b 11	30	II=18 b 78	19	II=19 b 99	21	I=30 b 105	6	+A(1)			
57	w	med.–20.3	XXIII=846 ckr 10	29	I=33 b 78	19	III=34 b 99	21	III=45 b 105	14	VI=55 b 110	28	VI=50 b 24	14
57	w	(*Continued*)	+A(2)											
59	w	med.–24.4	VII=117 ckr 10	30	III=30 b 78	19	IV=30 b 99	21	IV=39 b 105	14	VII=54 b 110	28	VI=50 b 24	14
59	w	(*Continued*)	VI=50 b 125	18	V=51 b 26	16	V=50 s 156	24	VI=52 s 28	14	VI=54 s 25	12	VIII=60 s 158	11
59	w	(*Continued*)	IX=73 s 106	5	+A(7)									
58	w	large 25.8–27.5	CXCI=7028 ckr 10	74	VIII=64 b 27	14	VI=54 b 92	24	VII=50 b 24	14	VI=55 b 125	18	VII=65 b 26	16
58	w	(*Continued*)	IV=63 s 156	24	VII=55 s 28	14	VI=60 s 25	12	IX=67 s 158	11	IX=83 s 25	10	XI=88 s 185	11
58	w	(*Continued*)	VIII=100 s 38	8	XXIV=229 s 109	7	XXXI=337 s 178							

TABLE 1.—*Continued.*

No.	Species	Weight in Grams	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days
61	g	16.9	XXVII=81 s 60	22	VI=69 b 82	3	+?							
63	g		XXII=196 s 60	4	+A(1)									
64	g	20.0	XX=270 s 32	18	V=69 b 82	4	+A(1)							
65	g	13.0	CV=315 s 32	18	IV=69 b 82	20	XII=66 b 99	3	+A(1)					
71	w	27.7	XI=97 s 68	14	IV=63 b 82	3	+A(1)							
72	w	18.0	XI=91 s 68	14	I=51 b 82	20	IX=52 b 96	6	+?					
73	w	27.0	XX=116 s 68	14	IV=52 b 82	20	IX=54 b 96	21	V=66 b 105	14	IX=63 b 110	3	+A(3)	
74	w	27- 25.8	IX=102 s 68	59	VII=61 b 27	14	VI=55 b 92	24	VI=49 b 24	14	VII=53 b 125	4	+A(5)	
78	w	large-25.4	XX=261 ckr 107	3	+A(1)									
79	w	large-21.8	XXV=366 ckr 107	48	VI=56 b 27	14	VII=48 b 92	24	VI=41 b 24	14	VII=45 b 125	18	V=49 b 26	6
79	w	(*Continued*)	+A(2)											
80	g	large-15.6	XXIII=235 ckr 107	3	+A(1)									
81	w	large	XXV=379 ckr 107	48	V=52 b 27	14	V=49 b 92	20	+?					
83	w		I=4 b 14	2	+?									
84	w	23.9	I=8 b 14	37	I=16 b 105	14	IV=28 b 110	28	V=37 b 24	14	IV=44 b 125	18	IV=45 b 26	16
84	w	(*Continued*)	IV=52 s 156	3	+A(5)									

85	w	20.0	I=8 b 14	37	I=15 b 105	14	II=25 b 110	3	+A(3)				
86	w	25.5	I=8 b 14	37	II=18 b 105	14	III=24 b 110	3	+A(3)				
87	w		I=8 b 14	1	+ ?								
88	w		I=8 b 14	3	+ ?								
89	w	17.0	I=6 b 14	6	+ ?								
90	w	26.0	II=28 l 3	33	III=41 l 19	13	V=56 l 111	3	+A(3)				
91	w	23.2	II=18 l 3	33	III=32 l 19	13	V=45 l 111	5	+A(3)				
92	w	25.0	II=12 l 3	33	II=20 l 19	13	VII=38 l 111	3	+A(3)				
93	w	28.0	III=34 l 3	33	IV=46 l 19	13	VIII=65 l 111	4	+A(3)				
94	w	21.0	III=27 l 3	32	+ ?								
95	w	24.2	XXV=340 ckr 147	29	VI=52 b 27	14	VII=48 b 92	3	+A(4)				
96	w	22.0	XX=216 ckr 147	3	+A(1)								
97	w	25.5–26.7	XX=268 ckr 147	29	V=49 b 27	14	III=34 b 92	26	IV=34 b 24	14	V=39 b 125	5	+A(5)
98	w	23.0	XX=246 ckr 147	2	+A(1)								
99	w	21.0	XXVIII=390 ckr 147	4	+A(1)								
113	w	18 –13.0	I=9 b 95	5	+ ?								
114	w	18.5–14.6	I=12 b 95	3	+ ?								
115	w	15.5–12.8	I=9 b 95	3	+A(5)								
116	w	20 –15.1	II=14 b 95	2	+ ?								

TABLE 1.—*Continued.*

No.	Species	Weight in Grams	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days
117	w	18.2–18.2	I=10 b 95		I=7 b 108	26	I=9 b 26	16	I=8 s 156	24	I=7 s 28	14	I=8 s 25	12
117	w	(*Continued*)	I=9 s 158	11	II=30 s 106	10	VI=48 s 185	11	VI=65 s 38	8	XXV=219 s 109	7	XXXI=339 s 178	2
117	w	(*Continued*)	+A(1)											
118	w	16.2–17.1	I=12 b 95	26	I=10 b 108	4	+A(3)							
119	w	18.1–23.9	II=10 b 95	27	II=12 b 108	26	I=12 b 26	16	I=12 s 156	24	I=12 s 28	14	I=14 s 25	12
119	w	(*Continued*)	II=12 s 158	3	+A(4)									
120	w	18.5–15.6	II=13 b 95	3	+A(5)									
121	w	22.3–20.8	III=18 b 95	3	+A(5)									
122	w	20.7–16.4	I=16 b 95	3	+A(5)									
125	w	22.3–21.6	II=9 b 118	4	+A(4)									
126	w	15.7–18.7	I=8 b 118	22	I=7 b 26	16	I=7 s 156	3	+A(5)					
127	w	13.2–13.7	I=8 b 118	4	+A(4)									
128	w	18.3–19.0	I=10 b 118	3	+A(4)									
129	w	23.5–24.6	I=9 b 118	3	+A(4)									
131	w	19.5–19.5	I=6 b 125	3	+A(5)									
132	w	20.7–14.9	I=7 b 125	4	+?									

133	w	18.8–26.6	I=6 b 125	18	I=6 b 26	16	I=6 s 156	24	I=6 s 28	14	I=4 s 25	12	II=7 s 158	11
133	w	(*Continued*)	III=34 s 106	10	VI=46 s 185	11	VI=53 s 38	8	XVI=193 s 109	3	+A(7)			
135	w	12.7–18.9	I=4.5 b 125	18	I=5 b 26	16	I=5 s 156	24	I=6 s 28	14	I=7 s 25	12	I=7 s 158	11
135	w	(*Continued*)	V=37 s 106	3	+A(7)									
136	w	16.5–21.0	I=6 b 125	4	+A(5)									
137	w	15.3–17.1	I=4 b 125	6	+A(5)									
138	w	15.9–17.8	II=6 b 125	3	+A(5)									
139	w	18.2–19.2 16.3	I=3 b 74	20	I=4.5 l 79	14	I=5 s 84	19	I=6 s 28	15	I=6 s 25	12	I=6 s 158	11
139	w	(*Continued*)	IV=40 s 62	10	VI=64 s 185	11	IX=127 s 38	8	XXI=236 s 109	5	+A(7)			
140	w	20.5–24.9 20.6	I=4 b 74	20	I=4 l 79	14	I=4 s 84	19	I=4 s 28	15	I=4 s 25	12	I=4 s 158	11
140	w	(*Continued*)	II=31 s 62	2	+A(1)									
141	w	20. –25.5 22.9	I=4 b 74	20	I=4 l 79	14	I=4 s 84	19	I=3 s 28	15	I=3 s 25	12	I=3 s 158	11
141	w	(*Continued*)	IV=51 s 62	3	+A(1)									
142	w	19.6–20.4 17.4	I=5 b 74	20	I=5 l 79	14	I=5 s 84	19	I=6 s 28	4	+A(1)			
143	w	19.5–20.6 18.7	I=5 b 74	20	I=6 l 79	14	I=7 s 84	19	I=7 s 28	15	I=6 s 25	12	I=6 s 158	11
143	w	(*Continued*)	IV=40 s 62	3	+A(1)									
144	w	19 –20.8 20.7	I=2 b 74	20	I=4 l 79	14	I=4 s 84	19	I=4 s 28	3	+A(1)			
145	w	17.4–16.9 16.2	I=3 b 74	20	I=3.5 l 79	3	+A(3)							
147	w	19.3 19.0	I=3+ b 74	20	I=9 l 79	14	I=7 s 84	19	I=6 s 28	15	+ ?			

TABLE 1.—*Continued.*

No.	Species	Weight in Grams	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days
148	w	10.7–27.7 18.8	I=3.5 b 74	20	I=4.5 l 79	14	I=4 s 84	19	I=4 s 28	15	I=3.5 s 25	12	I=3+ s 158	11
148	w	(*Continued*)	V=47 s 62	4	+A(1)									
149	w	19.8–20.2 19.8	I=5 b 74	20	I=6 l 79	3	+A(3)							
150	w	27.7–24.5	I=10 s 149	30	I=10 s 28	22	I=9 s 35	15	I=9 s 62	11	I=9 s 184	10	I=9 s 38	8
150	w	(*Continued*)	I=8 s 109	7	I=9 s 178									
152	w	23.4	I=9 s 149	29	I=8 s 28	22	I=9 s 35	15	I=10 s 62	11	I=9 s 184	10	I=11 s 38	8
152	w	(*Continued*)	II=14 s 109	7	I=13 s 178									
153	w	23.8–23	I=11 s 149	30	I=12 s 28	22	I=15 s 35	3	+A(3)					
154	w	21.2	I=10 s 148	7	+A(2)									
155	w	20.0–22.7	I=7 s 149	30	I=6 s 28	22	I=6 s 35	15	I=6 s 62	11	I=9 s 184	10	I=15 s 38	8
155	w	(*Continued*)	III=13 s 109	7	I=15 s 178	2	+A(1)							
156	w	20.3–20.1	I=9 s 149	6	+A(4)									
158	w	29 –25.5	I=6 s 149	30	I=6 s 28	22	I=7 s 35	4	+A(3)					
159	w	23.7–24.5	I=6 s 149	30	I=5 s 28	22	I=15 s 35	4	+A(3)					
160	w	20	I=6 s 149	30	I=5 s 28	22	I=12 s 35	15	II=12 s 62	11	I=12 s 184	10	I=12 s 38	8
160	w	(*Continued*)	II=13 s 109	7	I=12 s 178									

161	g		I=10 b 26	21	I=7 s 84								
162	g	19.1	I=21 b 26	3	+A(2)								
163	g		II=21 b 26	21	I=9 s 84								
165	g	13.7	I=26 b 26	3	+A(2)								
166	g		I=14 b 26	21	I=6 s 84								
168	g	13.6	I=10 b 26	21	II=8 s 84	2	+A(6)						
169	g	12.5	I=10 b 26	3	+A(2)								
171	w	13.3–11.8	III=36 s 31	6	+?								
172	w	19.0–20.0	IV=48 s 31	17	VI=52 s 143	9	XIV=89 s 187	8	XXIII=200 s 183	9	XXVII=282 s 109	7	XXXVI=419 s 178
173	w	18.5–21.0	V=36 s 31	4	+A(4)								
175	w	19.7	II=42 s 31	17	VIII=63 s 143	9	XI=80 s 187	8	XXIX=175 s 183	9	XXXIV=316 s 109	5	+A(1)
176	w	20.9–22.7	IV=37 s 31	17	VIII=67 s 143	9	XIV=109 s 187	3	+A(3)				
177	w	18.5–19.6	IV=39 s 31	17	VII=56 s 143	9	XVIII=176 s 187	8	XXVI=249 s 183	3	+A(5)		
179	w	19.7–25.6	IV=47 s 31	17	VII=59 s 143	9	XIII=102 s 187	3	+A(3)				
181	w	20.5–22.7	V=36 s 31 VI=105 2 ag b 31	3	+A								
182	w	17.2–18.2	III=39 s 31 IV=138 2 ag b 31	22	VII=62 s 22	8	XXII=193 s 176	8	XXIV=248 s 101	8	XLIII=340 s 133		

TABLE 1.—*Continued.*

No.	Species	Weight in Grams	Dose in Micromillimeters	Intervals between Doses—Days	Dose in Micromillimeters	Intervals between Doses—Days	Dose in Micromillimeters	Intervals between Doses—Days	Dose in Micromillimeters	Intervals between Doses—Days	Dose in Micromillimeters	Intervals between Doses—Days	Dose in Micromillimeters	Intervals between Doses—Days
183	w	18.5–22.0	III=42 s 31 IV=120 2 ag b 31	22	VIII=74 s 22	8	XIX=201 s 176	4	+A(4)					
184	w	19.2–19.4	V=46 s 31 IV=144 2 ag b 31	22	VI=59 s 22	3	+A(2)							
185	w	15.6–16	II=45 s 31 VII=198 2 ag b 31	22	XI=64 s 22	2	+A(2)							
186	w	18.8–17.8	VII=53 s 31 VII=180 2 ag b 31	22	VIII=64 s 22	8	XXI=179 s 176	8	XLVI=254 s 101	4	+A(6)			
187	w	23.5–19.2	V=50 s 31 VIII=165 2 ag b 31	22	VIII=96 s 22	3	+A(2)							

increase in weight during confinement. Some instances will be noted where mice succumbed to doses less than or equal to those which they had previously withstood.

So while the possibility of the acquirement of some artificial immunity cannot be excluded, all the variations noted can be explained equally as well or better by reference to the natural resistance of the animal.

As regards hypersensitization the evidence is also negative. In groups 113, 125, 131, 139, and 150 the same relatively small dose was repeated through many inoculations with especial reference to the determination of possible hypersensitizing. Here there was considerable mortality following the first inoculations, due presumably to the weeding out of the more susceptible individuals. Then followed a relatively slow rate of mortality with some individuals surviving many successive doses. Four out of nine members of group 150 survive seven inoculations, and in other groups also, where nearly constant doses of relatively larger size were given, some members survive many successive doses. The final infection and death of mice in these groups can be fully explained, it seems to me, by the possibility of doses of increased virulence, or by a falling off in the condition of the animal, without resorting to the assumption of hypersensitizing. For the relation between the number of the dose and the mortality see Table 5.

In Table 2 are grouped the results of experiments having the same aim as those of Table 1. Here spores are inoculated instead of fresh material. The spores, except in a few instances indicated in the table, were formed in the first culture from the animal, and were grown in a mixture of the culture medium and blood or organ extract from the infected animal. In the earlier experiments hanging drop cultures and a medium composed of a mixture of broth or salt solution with material from the infected animal were used. In later experiments blood or portions of organs were placed on peptone-free agar. Spores were in nearly all cases grown at 37° C. and inoculated soon after they were ripe, usually within 30 hours after planting. On one inoculation date spores dried for 17 days but still viable were inoculated. See * in Nos. 5, 6, 47, 48, and 49 in the table.

Spores were inoculated free after the dissolution of the mother cell,

TABLE 2.

MICE INOCULATED WITH VIRULENT SPORES.

Sp.=spore. Sp.+8=spore dose mixed with 8 micromillimeters of culture. Symbols otherwise as in Table 1.

No.	Species	Weight in Grams	Dose	Intervals between Doses—Days	Dose	Intervals between Doses—Days	Dose	Intervals between Doses—Days	Dose	Intervals between Doses—Days	Dose	Intervals between Doses—Days	Dose	Intervals between Doses—Days	Dose	Intervals between Doses—Days	Dose	Intervals between Doses—Days
5	w		1 sp b 1	7	2 sp b 16	8	3 sp b 15	8	5 sp* b 10	31	5 sp b 53	39	6 sp b 14	5	+A(2)			
6	w	21.4	1 sp b 1	7	4 sp b 16	8	8 sp b 15	8	9 sp* b 10	31	16 sp b 53	39	21 sp b 14	50	10 sp s 110	28	13 sp l 24	15
6	w	(*Continued*)	15 sp s 125	23	20 sp+40 s 79	13	24 sp l 84	21	29 sp+75 s 28	14	34+102 l 25	13	42 sp s 158	10	50 sp l 62	3	+A(1)	
7	w	med.	2 sp b 2	4	+A(2)													
8	w	med.	4 sp b 2	4	+A(2)													
9	w	med.	20 sp b 2	3	+A(2)													
10	w	med.	35 sp b 2	2	+A(2)													
11	w	med.	3 sp b 2	12	6 sp b 15	39	12 sp b 53	3	+A(3)									
12	w	med.–19.0	14 sp b 2	12	20 sp b 15	39	30 sp b 53	39	30 sp b 14	37	+ ?							
15	w	med.	4 sp b 10	9	+A(3)													
20	w	23.7	1 sp b 7	47	3 sp b 53	89	5 sp s 110	3	+A(4)									
21	w		1 sp b 7	11	+ ?													

22	w	29.4	1 sp b 7	47	2 sp b 53	38	3 sp b 14	50	4 sp s 110	28	5 sp l 24	15	6 sp s 125	23	7 sp s 79	13	8 sp l 84	21
22	w	*(Continued)*	9 sp s 28	14	10 sp+12 l 25	13	11 sp s 158	10	12 sp l 62	7	+A(1)							
23	w		2 sp b 7	47	2 sp b 53	89	4 sp s 110	28	5 sp l 24	15	6 sp s 125	23	7 sp+3 s 79	13	8 sp l 84	21	9 sp s 28	14
23	w	*(Continued)*	10 sp+12 l 25	13	11 sp s 158	10	12 sp l 62	11	13 sp s 184	10	14 sp+9 l 38	8	15 sp l 109					
42	w	26.3	2 sp b 13	35	5–6 sp b 53	38	6 sp b 14	36	10 sp b 105	15	20 sp st 111	27	14 sp+27 l 24	4	+A(2)			
43	w	26.5			3 sp b 13	73	7 sp b 14	36	7 sp+6 b 105	15	13 sp st 111	27	15 sp l 24	15	20 sp+9 s 125	23	24 sp+20 s 79	4
43	w	*(Continued)*	+A(3)															
44	w	21.0	4 sp b 13	43	9 sp b 14	3	+A(2)											
47	w	28.0	15 sp* b 10	32	6 sp b 53	13	10 sp b 105	15	13 sp st 111	27	15 sp+6 l 24	15	15 sp s 125	23	20 sp+30 s 79	13	25 sp+50 l 84	21
47	w	*(Continued)*	30 s+150 s 28	14	40 s+120 l 25	13	45 sp s 158	10	56 sp	21	55 sp+100 l 38	8	117 sp l 109					
48	w	24.1	4 sp* b 10	32	9 sp b 53	74	10 sp b 105	15	12 sp+6 st 111	27	24 sp+30 l 24	15	29 sp+12 s 125	23	35 sp s 79	13	30 sp l 84	21
48	w	*(Continued)*	40 sp+120 s 28	14	46 sp+120 l 25	3	+A(1)											
49	w	20.0	1 sp* b 10	106	7 sp b 105	15	12 sp st 111	2	+A(4)									

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* Dried spores.

or, especially during later inoculations, taken when still in chains. It was found exceedingly difficult to take up loose spores when isolated in hanging drops of liquid media, since, once in contact with the layer of surface tension at the bottom of the droplet, they could scarcely be separated from it. The pipette draws off the liquid, while the spore follows the surface tension layer to the cover glass and remains there. In a large variety of bacilli and spores manipulated by this technic I have found the above mentioned characteristic shown in any marked degree by none except the anthrax spore and the tubercle bacillus. In some cases I have partly overcome the difficulty by using gelatin, soft agar, or some other semi-solid medium in place of salt solution or broth. Again, if the anthrax spore or tubercle bacillus be taken up soon after isolation, while still suspended above the layer of surface tension and still exhibiting the Brownian movement, there is little difficulty in making them enter the pipette. Here I have found the double pipette holder of service, the bacillus or spore being taken up by a second pipette soon after its isolation by the first.

In some cases, indicated in the tables, filaments not containing spores were inoculated also. The amounts were small, and, considering that the threads were from a relatively old culture and probably somewhat degenerate, it is not likely that they had any effect on infection. In practically all mice of the spore series the dose was made to increase gradually. In No. 22 and the greater part of the histories of Nos. 5 and 23 the most gradual increase possible is maintained, namely one spore at a time. The results of experiments given in Table 2 are substantially the same as those of Table 1 and give no conclusive evidence of immunization or hypersensitization. (Compare Table 5.)

Table 3 contains the results of experiments with fresh cultures. With the exception of Nos. 188–191 cultures were made on agar and the number of removes from the animal are indicated on the table. Under "2ag," which includes the greater number of cases, infected blood or portions of organs were placed on the lower portion of agar slants and allowed to grow over night at 37°. Then the water of condensation, containing actively growing threads, was washed over the still sterile upper part of the slant of the same tube and allowed to grow, usually five or six hours, until a layer of new growth had formed. This fresh growth was used for inoculation. Altho grown in the first tube from the infected animal, this is practically equivalent to the second agar culture. This procedure was followed in order to have filaments for inoculation that were living and rapidly growing.

In group 106 a few are included in which some spores were mixt with the vegative filaments and indicate a somewhat older culture.

It is seen in Table 3 that animals withstand a much larger initial dose of anthrax from culture than from infected tissues. This dosage was increased to over 2,000 units in group 100 before any animals became infected. Then No. 103, which, it will be noted, had lost considerable weight, succumbed. In order to test the resistance of the survivors fresh virulent material was substituted for culture material at the 12th inoculation and the dosage increased until all had yielded. On comparing these lethal doses with those found under groups 171 and 181 of Table 1, it will be seen that the preliminary treatment with culture material had conferred very little, if any, immunity against fresh material, altho possibly these mice had attained some immunity against culture material.

In Nos. 188–191 virulent material was sown in hanging drops in a medium of salt solution mixt with spleen pulp. The growth of 24 hours at room temperature was inoculated. The results show that bacilli, tho taken directly from the animal and allowed to increase in an extract of an infected organ, soon lose much of their power of infection.

Up to doses of 500 to 600 units filaments were measured separately, as in doses given in Table 1. In larger doses the quantity was estimated by measuring, in droplets containing a few threads, the units in one or two filaments of suitable size. The total number of units in the droplet was then estimated by using the measured filaments as a standard. A few of the larger doses in Table 1 were estimated in this way.

As regards the bearing these experiments have on the subject of infection, reference may be made, first, to Table 4. Here the infectivity of anthrax taken directly from an infected insect instead of from a mouse may be estimated. The material was obtained by inoculat-

TABLE 3.

MICE INOCULATED WITH MATERIAL FROM CULTURES.

ss=material grown in hanging drops in mixture of mouse serum and salt solution.

No.	Species	Weight in Grams	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days	Dose in Micro-millimeters	Intervals between Doses—Days
100	w	23.8	XII=184 5 ag	19	XXI=300 5 ag b	27	XI=393 3 ag l 93	23	XXXVI=540 4 ag s 24	13	XLVI=666 2 ag s 125	21	XXXII=817 2 ag k 162	16
100	w	(*Continued*)	1035 2 ag b 84	21	1325 2 ag s 28	14	1551 2 ag l 25	11	2000 2 ag l 153		no inoc.	22	XI=120 s 184	9
100	w	(*Continued*)	XXVII=235 s 183	8	XXX=390 s 109	3	+A(1)							
101	w	25.8–25.4	XV=331 5 ag	19	XXXIV=516 5 ag b	27	XVII=564 3 ag l 93	23	XXVII=609 4 ag s 24	13	L=708 2 ag s 125	21	XXXVI=822 2 ag k 126	16
101	w	(*Continued*)	990 2 ag b 84	21	1323 2 ag s 28	14	1575 2 ag l 25	11	2322 2 ag l 153	11	V=52 s 62	11	IX=146 s 184	9
101	w	(*Continued*)	XXXVII=291 s 183	3	+A(5)									
102	w	28.8–16.2	VI=180 5 ag	19	XIX=325 5 ag b	27	XVI=378 3 ag l 93	23	XXI=528 4 ag s 24	13	XXV=648 2 ag s 125	21	XXXVI=867 2 ag k 162	16
102	w	(*Continued*)	1062 2 ag b 84	7	+A(6)									
103	w	27.8–20.1	X=310 5 ag	19	XLI=618 5 ag b	27	XVII=720 3 ag l 93	23	XXVII=792 4 ag s 24	13	LXVII=897 2 ag s 125	21	LX=1008 2 ag k 162	16
103	w	(*Continued*)	1200 2 ag b 84	21	1400 2 ag s 28	14	1710 2 ag l 25	11	2160 2 ag l 153	3	+A(4)			
104	w	20.3–24.7	IX=243 5 ag	19	XXVIII=498 5 ag b	27	XX=510 3 ag l 93	23	XXVI=693 4 ag s 24	13	LXXI=777 2 ag s 125	21	LVI=918 2 ag k 162	16
104	w	(*Continued*)	1145 2 ag b 84	21	1515 2 ag s 28	14	1893 2 ag l 25	11	2280 2 ag l 153	11	VII=68 s 25	11	XVI=151 s 184	9
104	w	(*Continued*)	XXXV=346 s 183	3	+A(5)									

112	w	21	-16.9					XXV=664 3 ag l 56 + ?	32	XXII=600 2 ag s 24	13	XXVIII=702 2 ag s 125	21	XLVII=894 2 ag k 162	16
112	w	(*Continued*)		960 2 ag b 84	21	1320 2 ag s 28	3								
106	w			XXIII=305 4 ag l 56	33	48 sp 4 ag s 24	18	VIII=177 3 ag s 74							
107	w			34 sp 4 ag l 56	33	48 sp+132 4 ag s 24	18	VI=16 sp+123 3 ag s 74	70	+ ?					
108	w		24.3	XXXVIII=444 4 ag l 56	33	51 sp+147 4 ag s 24	3	+A(2)							
109	w			18 sp+18 4 ag l 56	33	32 sp 4 ag s 24	18	IX=31 sp+243 3 ag s 74							
110	w	21		XXVII=675 3 ag l 56	2	+A(2)									
111	w	21		XLVI=1077 3 ag l 56	3	+A(2)									
188	g			900 ss 62											
189	g			1233 ss 62											
190	g			750 ss 62											
191	g		8.3	690 ss 62	2	+A(1)									

ing cockroaches in the abdomen or leg by means of a fine-pointed capillary pipette. When large doses were given the animals usually succumbed to anthrax in 24 to 48 hours, whether kept at incubator or room temperature. It is observed that this material of insect origin is somewhat less infective for mice than that of mouse origin. (Compare Table 6.) The bacilli from cockroaches were, for the most part, capsulated as shown by staining and by observing them mounted

TABLE 4.
MICE INOCULATED WITH MATERIAL FROM INFECTED COCKROACHES.

No.	Species	Weight in Grams	Dose in Micromillimeters	Intervals between Doses—Days	
66	g	med.	XXXIX=624 ckr 54	2	+A(1)
67	g	med.	XXIV=453 ckr 54	2	+A(1)
68	g	med.	LXVI=1044 ckr 54	3	+A(1)
69	g	small	XXXIX=720 ckr 54	2	+ ?
70	w	large	LXXIX=1136 ckr 54	4	+A(1)
57	w	med.–20.3	XXIII=846 ckr 10	29	*
58	w	large–25.8	CXCI=7028 ckr 10	74	*
59	w	med.–24.4	VII=117 ckr 10	30	*
78	w	large–25.4	XX=261 ckr 107	3	+A
79	w	large–21.8	XXV=366 ckr 107	48	*
80	g	large–15.6	XXIII=236 ckr 107	3	+A
81	w	large	XXV=379 ckr 107	48	*
95	w	24.2	XXV=340 ckr 147	29	*
96	w	22.0	XX=216 ckr 147	3	+A
97	w	25.5–26.7	XX=268 ckr 147	29	*
98	w	23.0	XX=246 ckr 147	2	+A
99	w	21.0	XXVIII=390 ckr 147	4	+A

* For subsequent doses see Table 1.

in India ink. These results would indicate that it is not the capsule alone which determines the greater infectivity of bacilli of animal origin else we would expect the cockroach material to be as infective as the mouse.

Below No. 70 in this table are given a group of 12 taken from Table 1, the initial doses of which are repeated for comparison.

In Table 5 under each dose number is shown the total number of mice which reached this dose number, and the number and percentage dying of anthrax. Thus of the 68 which received a second dose, 10, or 15 per cent, died. Of the virulent vegetative material only doses of 100 μ or less are included, and in the spore series only doses of 30 spores or less. It will be noted that there is no tendency to an increase or decrease of mortality until the eighth dose is reached, when the few survivors show considerable resistance. This is the more noteworthy since the doses beyond the seventh inoculation were greater than 30 microns or nine spores. If the interpretation of results given above is accepted we have here the phenomenon sometimes observed in severe epidemics—the survival of the few exceptionally well endowed with natural resistance. If hypersensitiveness were being developed we would expect a progressive increase in the percentage mortality.

TABLE 5.

RELATION BETWEEN NUMBER OF DOSE AND NUMBER OF MICE DYING.

Doses 100 μ or less and 30 spores or less.

Number of Dose Virulent Vegetative	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number inoculated	83	68	55	35	30	23	19	10	5	4	3	3	2	0
Number dying	20	10	15	4	7	3	9	1	0	0	0	1	1	0
Percentage dying	24	15	27	11	23	13	47	10	0	0	0	33	50	0
Number of Dose Spore Series														
Number inoculated	18	13	12	9	8	8	5	5	4	3	3	3	1	1
Number dying	4	1	3	0	0	2	1	0	0	0	0	1	0	0
Percentage dying	22	7	25	0	0	25	20	0	0	0	0	33	0	0

In Table 6 the mouse numbers and the percentages dying of anthrax are arranged under doses of different magnitude instead of under dose number. Doses of all magnitudes are considered and results are arranged according to the source of the inoculation material. The smallest lethal dose was observed in No. 145, where the fatal dose, $3\frac{1}{2}$ μ, presumably one bacillus, was taken from the liver of an infected mouse. In another case (No. 7) the lethal dose was two spores. The increased mortality with the larger doses is observable. The relative infectivity of material from the different sources is also shown. Roughly estimated, material directly from the infected mouse is nearly 100 times as infectious as material from fresh cultures. The number receiving cockroach material is too small to form a basis for a numerical estimate, but this material is evidently somewhat less infectious for mice than material of mouse origin.

TABLE 6.
SIZE OF FATAL DOSES AND NUMBERS DYING AT EACH QUANTITY.
VEGETATIVE.

MICRO-MILLIMETERS	FRESH FROM INFECTED MICE			CULTURE			FRESH FROM INFECTED COCKROACH		
	Total No. Inoculated	No. Dying	Percentage Dying	Total No. Inoculated	No. Dying	Percentage Dying	Total No. Inoculated	No. Dying	Percentage Dying
1–5	41	3	7½						
6–10	88	17	19						
11–15	36	6	17						
16–25	18	5	27						
26–35	29	4	13						
36–50	63	15	23						
51–75	63	19	30						
76–100	11	3	27						
101–150	9	3	33				1		
151–200	7	2	28	3					
201–300	13	5	38	2			5	4	80
301–400	6	4	67	6			4	1	25
401–600	1	0	0	7			1	1	100
601–800				15	2	13	2		
801–1000				9			1		
1001–2000				16	2	12	2	2	
2001–				4	1	25	1		

Table 7 is similar to Table 6 except that spore doses are considered. Doses of all sizes are included.

TABLE 7.
SIZE OF FATAL DOSES AND NUMBERS DYING AT EACH QUANTITY.
Spores.

Dose No. of Spores	2	3–5	6–8	9–11	12–14	15–18	19–25	26–30	31–45	46–50	50–
Number inoculated	8	18	15	14	12	9		6	10	4	5
Number dying	1	3	1	1	4	2		1	1	2	1
Percentage dying	12	16	6	7	33	22		16	10	50	20

There seems to be no definite relation between mortality and the number of threads or segments composing a dose of given size. Summarizing the data furnished by the Roman numerals in Table 1 we find out of a total of 78 dying of anthrax, 25 received one thread in the lethal dose; 9 received two; 5, three; 6, four; 8, five; 5, six; 12, seven to fifteen; and 9, sixteen and above. Of 26 receiving fatal doses of 15 μ or less, 22 received but one thread.

As regards the virulence of the inoculated material, so far as it can be measured by the number of consecutive mouse passages, the following data, summarized from Table 1, are included in Table 8.

TABLE 8.
VIRULENCE, AS SUMMARIZED FROM TABLE 1.

Inoculation Material No. of Mouse Passages	Total No. Mice Inoculated	No Dying of of Anthrax	Percentage Dying of Anthrax
1	78	23	29
2	33	9	27
3	46	17	37
4	55	12	22
5	37	15	40
6	14	2	14
7	16	3	19

As regards the portion of the infected animal from which the inoculated material was taken, the following data are summarized from Table 1: Blood, total number of inoculations, 183, mortality from anthrax, 24 per cent; spleen, total inoculations 176, mortality 17 per cent; liver, total inoculations 23, mortality 26 per cent. The spleen furnished a larger proportion of the small doses than the other organs.

The intervals between the last dose and death of the mice in Tables 1, 2, 3, and 4 are given in Table 9. For comparison are also given the intervals following very large doses given to 23 mice not included in any of the above tables. These large doses were about $\frac{1}{10}$–$\frac{1}{20}$ cm. emulsion of organs or of cultures.

TABLE 9.

Intervals in Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Totals
Mice from tables	0	16	57	26	11	4	3	0	2	0	0	0	0	1	116
Mice receiving massive doses	14	5	1	2	0	0	0	0	1	0	0	0	0	0	23

A histological examination was made of about 85 mice. As a rule, spleen, liver, kidney, and, in a considerable number of cases, tissues taken from beneath the point of inoculation, were sectioned in paraffin and stained by the Weigert method. In by far the greater number of those which died of anthrax the usual distribution of bacilli was found in the organs; but in some the spleen, usually showing the largest number, had very few (in the spleen of No. 141 none could be found), while other organs, and especially the point of inoculation, showed them more abundantly. There seemed to be no relation between the numbers and distribution of bacilli and the dosage or other treatment of the animals, tho animals of the spore series seemed to show somewhat greater numbers in the organs.

Large masses of bacilli were often found in the tissues at the point of inoculation, and near these were frequently found collections of phagocytes. In the masses of phagocytes bacilli were often granular and apparently degenerate. This condition was found in animals which had received but one dose as well as in those which had received several and could not therefore be regarded as a sign of acquired resistance.

Sections were made of nearly all those which died of some cause apparently other than anthrax, and cultures were made from the organs of some doubtful ones. It is probable that, owing to the possibility of missing some small focus of infection, some of these animals should be included among those which died of anthrax, especially when the interval following the last dose was two to six days.

Considerable edema was observed at or near the point of inoculation of many at autopsy. This was found in those which had received but one dose, as well as in those which had received several, and so could have no relation to acquired immunity.

Considering the difference in size, the gray mice reacted about the same as the whites as regards infection and immunity. A guinea-pig inoculated with five spores died of anthrax after a five days' interval.

In conclusion is may be stated that these experiments give no ground for the assumption that any degree of immunity or hypersensitiveness can be conferred on the mouse by repeated minute doses of anthrax, either in the vegetative stage taken directly from the infected animal, in the form of virulent spores, or in the vegetative form somewhat attenuated by cultivation. The conditions imposed on these experiments, the combination of high virulence with high susceptibility, are severe; and results might be otherwise in the case of another animal or microorganism where either or both factors might be materially altered.

As regards the lethal dose of material from infected organs a bacillus $3\frac{1}{2}$ μ long was, in one case, found to be a fatal dose; and it is evident that a sublethal dose, under these conditions, does not exist for a very susceptible individual. Fresh virulent material from the mouse has for mice about 100 times the infectivity of material grown in the first agar or broth tube, and a somewhat greater infectivity than that of capsulated bacilli directly from infected cockroaches.